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Autoantibodies to the High-Affinity IgE Receptor in Patients with Asthma

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SUMMARY Autoimmune diseases have been implicated as a cause of intrinsic asthma; however, there is little data on the role of autoimmunity in the pathogenesis of asthma. The purpose of this study was to investigate circulating autoantibodies against the high-affinity IgE receptor FcεRI in patients with asthma. Seventy-eight patients with asthma and 32 healthy individuals as control subjects were included. All subjects were tested with basophil histamine releasing assay and immunoblotting to assess for the potential presence of receptor FcεRI autoantibodies. Of the 78 asthma patients total subjects, 25 (32.1%) had a positive by basophil histamine releasing assay and 23 (29.5%) by immunoblotting. Both of them were significant higher than the positive rate, 9.4% ($p < 0.05$) and 9.4% ($p < 0.05$), respectively. Our data demonstrated that aberrant autoantibodies against the high-affinity IgE receptor FcεRI were found in some patients with asthma implies that the autoimmunity may be one factor in intrinsic asthma pathogenesis.

Asthma is an incurable chronic inflammatory disease of the airways characterized by persistent eosinophilic mucosal inflammation, elevated serum IgE titers, mast cell hyperdegranulation, increased mucus production, and associated functional changes to the lung.[1] The prevalence and severity of asthma are rising, and asthma is now a global epidemic.[2] Considerable evidence suggests that molecular defects in Th lymphocyte immune regulation and multiple immune reactions contribute to the pathogenesis of asthma.[3-7] The other mechanisms apart from atopy remain to be clarified. Allergy and autoimmune disease are two potential outcomes of dysregulated immunity. Both are characterized by localized inflammation that leads to injury and/or destruction of target tissues. Until recently, it was generally accepted that the mechanisms that govern these disease processes are quite disparate. However, new discoveries suggest a possible pathogenetic linkage. Basophil granulocytes and tissue mast cells and their mediators play a role in the pathogenesis of asthma. Human basophils and mast cells (FcεRI+ cells) can be activated through immunological interaction with the IgE-FcεRI network. Anti-IgE autoantibodies are occasionally present even in normal donors and, more frequently, in a variety of allergic diseases such as chronic urticaria, atopic dermatitis and bronchial asthma, as well as in autoimmune disorders such as rheumatoid arthritis, lupus erythematosus and systemic sclerosis.[8] Therefore,

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to confirm the potential relationship or common mechanism, we have investigated the functional autoantibodies against the high-affinity IgE receptor FcεRI in patients with asthma.

MATERIALS AND METHODS

Subjects

Subjects with a cough for more than 3 weeks were recruited from the outpatient clinics of Guangzhou Institute of Respiratory Disease, China. Seventy-eight asthma patients exhibited one or more asthma symptoms, and their physical examinations were compatible with the definition of asthma according to the standard issued by the American Thoracic Society of the American Thoracic Society.[9] Thirty-two healthy individuals awaiting elective eye surgery were taken as controls. A healthy laboratory control was used for the basophil release assays. The study was approved by the First affiliated Hospital of Guangzhou Medical Collage Ethics Committee.

Functional anti–Fcε receptor assays[10]

Functional circulating autoantibodies to the IgE receptor were determined using purified IgG-mediated histamine release from basophils of nonatopic donors. Patient IgG was extracted from an aliquot of serum, following caprylic acid treatment in the presence of acetate buffer; dialyzed in phosphate-buffered saline solution by ammonium sulfate precipitation at 50% concentration; reconstituted to the original volume; and dialyzed extensively with phosphate-buffered saline solution. One nonallergenic adult, whose basophils were screened with anti-IgE for greater than 25% release, was used for the assay. Basophils in freshly collected whole blood samples were incubated with patient IgG diluted 50% in piperazine-1,4-bis (2-ethanesulphonic acid) (Sigma, St Louis, MO) containing 0.03 g/l human serum albumin and 2 ng/ml of recombinant interleukin 3 (PharMingen, Becton Dickinson, San Diego, CA) for 60 minutes at 37°C. The resulting histamine release was measured using a radioimmunoassay (Immuno-Biological Laboratories, Hamburg, Germany, and later Biosource, Nivelles, Belgium). A sample of IgG from a nonatopic individual was used in the histamine-release assay measured and subtracted to determine specific release. Patient IgG-stimulated histamine release was defined as a percentage of the total cellular histamine content, and it was considered positive if it was at least 16.5%, because the borderline histamine release was 5.0%–16.4% of total basophils histamine according to our previous study.[10] Dilutions of serum were also studied in the basophil-release assay for the detection of histamine-releasing titers. Spontaneous release in all experiments is less than 10%. The test result is considered positive only at a release of 16.5% or greater of the total cellular histamine content. This cutoff value was used for IgG- and serum factor–induced histamine release.

Detecting anti-FcεRI by immunoblotting

Purified recombinant FcεRIα was prepared as previously described by our group.[11] Once boiled and reduced with 2-mercaptoethanol (Kodak, Rochester, N.Y.), the sample was electrophoresed with 10% sodium dodecyl sulfate gels and blotted onto nitrocellulose membranes (Costar Scientific Co., Cambridge, Mass.). The membranes were blocked with 5% dry milk for 90 minutes and reacted with serum at a dilution of 1:200 because we predetermined that none of the control sera were positive at this dilution,[11] and some normal sera were positive when tested between 1:50 and 1:150. Bound IgG was detected with a goat ant-human IgG (Fc fragment-specifc alkaline phosphate conjugate, dilution 1:5,000) (Jackson ImmunoResearch Laboratories Inc., West Grove. Pa.). Antibody binding was visualized by incubating the membrane with BCIP/NBT phosphatase substrate (SABC Inc., China).

Statistical analysis

The statistical evaluation was performed using a software program (SPSS; SPSS Inc, Chicago, IL). The Chi-square test was used to compare the study and control group. A p value < 0.05 was set to determine level of significant difference.

RESULTS

The results of the FcεRI autoantibodies performed in 78 patients are given in Table 1. Twenty-five (32.1%) of 78 patients had positive autoantibody test results where histamine release from their baso-

Table 1 Presence of FcεRI autoantibodies*

	Asthma patients (n = 78)	Controls (n = 32)	X^2, p
Positive IgG FcεRI autoantibodies (histamine release, ≥ 16.5%)	25 (32.1%)	3 (9.4%)	5.01, $p < 0.05$
Positive IgG FcεRI autoantibodies by immunoblotting	23 (29.5%)	3 (9.4%)	4.03, $p < 0.05$

phils was at least 16.5% (range, 16.5%–60.0%). Mean histamine release in the positive group was 28.3%. Three of the 32 IgG samples from the controls had histamine-releasing activity, and it was above 9.4% of total histamine.

We performed immunoblots of 78 sera, and we observed a positive blot in 23 (29.5%) of them. Three of 32 (9.4%) controls also showed positive blots. We obtained a single protein band at about 60 kDa in all positive instances which were accordance with our previous reports.

DISCUSSION

Our findings provide further insight into asthma pathologic basis against the high-affinity IgE receptor FcεRI in patients with asthma. Among the patients with asthma, we found that autoantibodies against FcεI were observed in about 32% and 30% by basophil histamine releasing assay and immunoblotting, respectively. Those data suggest that autoantibodies against FcεRI may be involved in asthma. Asthma mechanisms apart from atopy remain to be clarified in the development of airway responsiveness and inflammation. Autoimmune disease has been implicated as a cause of intrinsic asthma.[12]

FcεRI is a multimeric surface receptor that is expressed exclusively as a tetramer on rodent cells, but exists as a tetramer or trimer on human cells. On mast cells and basophils, FcεRI is essential for IgE-mediated acute allergic reactions. Cross-linking of the IgE-loaded high-affinity IgE receptor by multivalent antigens results in mast cell activation and subsequent release of multiple proinflammatory mediators.[13] We had made studies on FcεRI and and found anti- FcεRI autobodies in some urticaria patients.[10-11] Taken together with this findings, it was thought that allergy and autoimmunity result from dysregulation of the immune system. The concomitant presentation of those conditions and the potential relationship or common mechanisms are revealed in some cases by looking at the key elements that regulate the immune response in both asthma and autoimmune conditions: mast cells, antibodies, T cells, cytokines, and genetic determinants. The autoantibodies against FcεRI or IgE may be involved in the pathogenesis of asthma.[14-16] We found that 30-32% of asthma patients revealed positive autoantibodies against FcεRI suggesting that autoimmunity may be involved in the pathogenesis of asthma.

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